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(54) Peptides and compositions thereof for improvement of glycaemic management in a mammal

(57) A peptide having 12 to 60 amino acids and including (a) a sequence of SEQUENCE ID NO: 11, or (b) a fragment of SEQUENCE ID NO: 11 that includes the sequence of SEQUENCE ID NO: 1 or 5, is described for use in improving glycemic management in a mammal. A composition, for example a food product, that includes substantially all of the peptides of SEQUENCE ID NO:'s 1 to 11, that is capable of reducing post-prandial blood glucose levels, and increasing insulin secretion in humans, is also described.

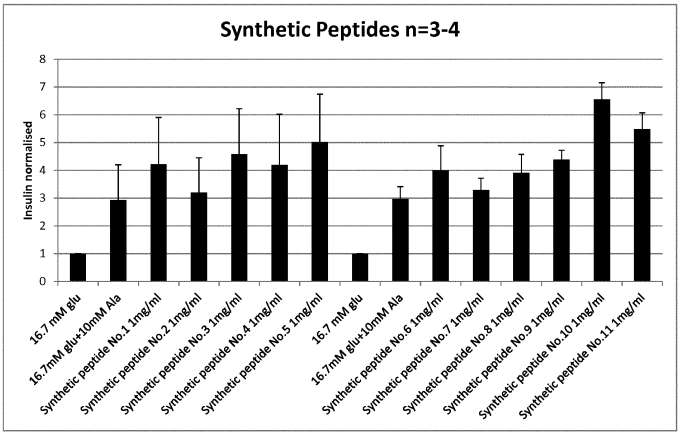

Figure 7B

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Description

Background to the Invention

[0001] High blood sugar levels (hyperglycaemia) are increasingly prevalent due to the spiralling levels of obesity worldwide. The WHO estimates that more than 1.4 billion adults over the age of 20 are overweight/obese, whereas one third of adults in the US and 108 million people in Asia have been diagnosed with diabetes. Hyperglycaemia is now as important as hypertension and hypercholesterolaemia, which is leading to increased awareness among health professionals and health-conscious consumers who demand products to aid post-prandial glucose levels.

[0002] It is an object of the invention to overcome at least one of the above-referenced problems.

Statements of Invention

[0003] Broadly, the Applicants have identified a number of peptides that have utility in improving glycemic management/status in mammals, in particular lowering post-prandial blood glucose levels and/or increasing insulin secretion following oral ingestion. In particular, the Applicant has identified that the peptides, and a composition comprising the peptides, can increase insulin secretion in-vitro in pancreatic beta cells (Figs. 7A-7C). The Applicant has also shown that acute treatment with a composition comprising the peptides had a glucose lowering effect during a glucose tolerance test in ob/ob mice, and caused a decrease in liver fat content in the mice (Figs 8 and 9). Using a diet induced obesity model, animals treated for 12 weeks with a composition comprising the peptides in the context of high fat feeding improved glucose clearance during a glucose tolerance test (GTT) compared to high fat feeding alone (Fig. 11). Additionally, the Applicant has demonstrated that a composition comprising peptides of the invention when administered to humans in a clinical study along with a carbohydrate meal causes a lowering of blood glucose levels and an increase in insulin levels (Figs 1 and 2).

[0004] The peptides are, or comprise, SEQUENCE ID NO: 11 below, and bioactive fragments thereof that typically include the sequence of SEQUENCE ID NO: 1 or 5, or both.

YPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSK (SEQUENCE ID NO: 11)

[0005] Specific bioactive fragments include SEQUENCE ID NOs 1 to 10 below.

HQPHQPLPPTVMFPPQSVL (SEQUENCE ID NO: 1)
HQPHQPLPPTVMFPPQSVLSLSQSK (SEQUENCE ID NO: 2)
LQSWMHQPHQPLPPTVMFPPQSVL (SEQUENCE ID NO: 3)
LQSWMHQPHQPLPPTVMFPPQSVLSLSQSK (SEQUENCE ID NO: 4)
PPQSVLSLSQSK (SEQUENCE ID NO: 5)
MHQPHQPLPPTVMFPPQSVL (SEQUENCE ID NO: 6)
MHQPHQPLPPTVMFPPQSVLSLSQSK (SEQUENCE ID NO: 7)
SWMHQPHQPLPPTVMFPPQSVL (SEQUENCE ID NO: 8)
SWMHQPHQPLPPTVMFPPQSVLSLSQSK (SEQUENCE ID NO: 9)
YPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVL (SEQUENCE ID NO: 10)

[0006] In one aspect, the invention relates to a peptide including (a) a sequence of SEQUENCE ID NO: 11, or (b) a bioactive fragment thereof, that preferably includes the sequence of SEQUENCE ID NO: 1 or 5 (hereafter "peptide of the invention").

[0007] In another aspect, the invention relates to a peptide of the invention for use in improving glycemic management and aspects of the metabolic syndrome in a mammal.

[0008] Thus, the invention relates to one or more of:

- a peptide of the invention for use in lowering plasma blood glucose levels, especially post-prandial blood glucose levels, in a mammal;
- a peptide of the invention for use in treating or preventing hyperglycaemia, in a mammal;
- a peptide of the invention for use in increasing post prandial insulin secretion, , in a mammal;
- a peptide of the invention for use in regulating glucose homeostasis, in a mammal;
- a peptide of the invention for use in treating or preventing or attenuating insulin resistance, in a mammal;
- a peptide of the invention for use in lowering plasma cholesterol levels, in a mammal;
- a peptide of the invention for use in treating or preventing or lowering liver fat content, in a mammal; or
- a peptide of the invention for use in promoting an anti-inflammatory response in a mammal;.

[0009] In another aspect, the invention also relates to a composition comprising a plurality of different peptides of the invention, for example at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 different peptides of the invention. Preferably, each peptide of the invention includes a sequence of SEQUENCE ID NO: 11, or a fragment thereof that include the sequence of SEQUENCE ID NO: 1 or 5.

[0010] In a further aspect, the invention also relates to a composition comprising substantially all of peptides of SEQUENCE ID NO's: 1 or 11. Suitably, the composition is a hydrolysate of a bovine casein product.

[0011] In another aspect, the invention relates to a composition of the invention for use in improving glycemic management in a mammal. In particular, the invention relates to a composition of the invention, for use in:

- lowering plasma blood glucose levels, especially post-prandial blood glucose levels, in a mammal;
- treating or preventing hyperglycaemia, in a mammal;
- increasing post prandial insulin secretion, in a mammal;
- treating or preventing regulating glucose homeostasis, in a mammal;
- treating or preventing or attenuating insulin resistance, lowering plasma cholesterol levels, in a mammal;
- treating or preventing or lowering liver fat content, in a mammal; or
- promoting an anti-inflammatory response in a mammal;.

[0012] In a further aspect, the invention relates to a food product comprising a composition of the invention. Typically, the food product is a Food for Specific Medicinal Purposes (FSMP).

[0013] In another aspect, the invention relates to a food product of the invention for use in improving glycemic management in a mammal. In particular, the invention relates to a food product of the invention, for use in:

- lowering plasma blood glucose levels, especially post-prandial blood glucose levels, in a mammal;
- treating or preventing hyperglycaemia in a mammal;
- increasing post prandial insulin secretion, in a mammal;
- regulating glucose homeostasis, in a mammal;
- reducing insulin resistance in a mammal;
- lowering plasma cholesterol levels in a mammal;
- treating or preventing lowering liver fat content in a mammal; or
- promoting an anti-inflammatory response in a mammal.

[0014] In a further aspect, the invention relates to a composition or peptide of the invention for use in preventing or treating a condition selected from a metabolic disorder or obesity. Thus, the invention also relates to a method for the prevention or treatment of a metabolic disorder in a human, comprising the step of administering to the human a therapeutically effective amount of the composition or peptide of the invention.

[0015] In a further aspect, the invention provides a pharmaceutical composition comprising a peptide or composition of the invention in combination with a suitable pharmaceutical carrier.

[0016] The invention provides a bovine casein product derived hydrolysate.comprising a plurality of different peptides of the invention, for example at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 different peptides of the invention. Suitably, at least 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% (w/w) of the hydrolysate comprises peptides of the invention. Typically, the hydrolysate of the invention is obtained by performing hydrolysis on a bovine casein product using a gastrointestinal enzyme preparation between the temperature range of 30°C to 60°C.

[0017] The composition of the invention may comprise a hydrolysate of the invention.

[0018] The invention also relates to a method of making a bovine casein product derived hydrolysate of the invention comprising the steps of providing a bovine casein derived product, performing enzymatic hydrolysis on the bovine casein derived product using a gastrointestinal protease preparation at a temperature of 30°C to 60°C for a suitable period of time.

[0019] The bovine-casein product typically comprises a casein salt, examples of which will be well known to those skilled in the art and include sodium caseinate. Typically, the bovine casein product (and/or the casein hydrolysate) is substantially free (i.e. less than 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, 0.1% (w/w) of other milk proteins, for example whey proteins.

[0020] Typically, the ratio of protease to casein substrate (w.w) is from 0.1 to 1.0%, preferably 0.2 to 2.0 %.

[0021] Suitably, hydrolysis of the casein substrate with protease preparation is carried out at a temperature of from 30°C to 60°C.

Definitions

[0022] In this specification, the term "improving glycemic management" should be understood to mean lowering plasma blood glucose levels, especially post-prandial blood glucose levels, treating or preventing hyperglycaemia, increasing

post prandial insulin secretion, regulating glucose homeostasis, reducing insulin resistance. The term "improving metabolic health" should be understood to mean lowering plasma cholesterol levels, lowering liver fat content, or promoting an anti-inflammatory response- or any combination thereof.

[0023] In this specification, the term "mammal" should be understood to mean a higher mammal, especially a human. Typically, the human has a disorder selected from a metabolic disorder such as diabetes, or obesity.

[0024] The term "peptide" used herein refers to a polymer composed of up to 60 amino acid monomers via peptide bond linkage. Peptides (including variants and fragments thereof) of and for use in the invention may be generated wholly or partly by chemical synthesis or by expression from nucleic acid. For example, the peptides of and for use in the present invention can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods known in the art (see, for example, J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984). When necessary, any of the peptides employed in the invention can be chemically modified to increase their stability. A chemically modified peptide or a peptide analog includes any functional chemical equivalent of the peptide characterized by its increased stability and/or efficacy in vivo or in vitro in respect of the practice of the invention. The term peptide analog also refers to any amino acid derivative of a peptide as described herein. A peptide analog can be produced by procedures that include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods that impose conformational constraint on the peptides or their analogs. Examples of side chain modifications include modification of amino groups, such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidation with methylacetimidate; acetylation with acetic anhydride; carbamylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulfonic acid (TNBS); alkylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxa-5'-phosphate followed by reduction with $NABH_4$. The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal. The carboxyl group may be modified by carbodiimide activation via o-acylisourea formation followed by subsequent derivatization, for example, to a corresponding amide. Sulfhydryl groups may be modified by methods, such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of mixed disulphides with other thiol compounds; reaction with maleimide; maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulfonic acid, phenylmercury chloride, 2-chloromercuric-4-nitrophenol and other mercurials; carbamylation with cyanate at alkaline pH. Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides. Tryosine residues may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative. Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate. Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. Peptide structure modification includes the generation of retro-inverso peptides comprising the reversed sequence encoded by D-amino acids.

[0025] In this specification, the term "peptide of the invention" means a peptide including a sequence of SEQUENCE ID NO: 11, or a bioactive fragment thereof. The term "bioactive fragment thereof" as applied to SEQUENCE ID NO: 11, means a fragment of SEQUENCE ID NO: 11 having at least 10 amino acids and which is capable of increasing insulin secretion from pancreatic beta cells compared with a control in the *in-vitro* test described below with reference to Fig. 7B. Examples of bioactive fragments of SEQUENCE ID NO: 11 have at least 12 amino acids and include the sequence PPQSVLSLSQSK (SEQUENCE ID NO: 5) or the sequence HQPHQPLPPTVMFPPQSVL (SEQUENCE ID NO: 1). Specific examples of bioactive fragments of SEQUENCE ID NO: 11 include the sequence of SEQUENCE ID NO'S 1 to 11 (Fig. 7B).

[0026] The compositions of the invention comprises a mixture of different peptides comprising at least 2 peptides, for example 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 different peptides, in which the peptides are selected from SEQUENCE ID NO: 11 or a bioactive fragment thereof. In one embodiment, the composition comprises substantially all of the peptides of SEQUENCE ID NO: 1 to 11, for example 8, 9 or 10 of the peptides. The term "composition comprising substantially all of peptides of SEQUENCE ID NO'S 1 to 11" should be understood to mean a composition comprising at least 8, 9 or 10 of the peptides. The composition may also include additional peptides, polypeptides or proteins, or other components, for example a poorly digestible carbohydrate.

[0027] The composition may be prepared by hydrolysing a bovine casein product, for example sodium caseinate, using a gastrointestinal protease preparation at a suitable temperature and for a sufficient period of time to provide a hydrolysate comprising at least two of the peptides of SEQUENCE ID NO: 1 to 11. The term "bovine casein product" as used herein should be understood to mean products derived from bovine milk that are predominantly composed on

casein protein, for example rennet caseinsates and acid caseinates (sodium, calcium and potassium caseinates). The term "gastrointestinal protease preparation" as employed herein should be understood to mean a protease preparation that includes trypsin, chymotrypsin, and elastase. Typically, the hydrolysis is carried out at 30°C-60°C.

[0028] In one embodiment, the composition of the invention is a casein-derived hydrolysate comprising a range of peptides of SEQUENCE ID NO's: 1 to 11, hereafter referred to as "LFC24". The composition of the invention is typically capable of lowering post-prandial blood glucose levels, increasing post-prandial insulin secretion levels, or both, when administered to an adult human according to the conditions of the clinical study described below.

[0029] The invention also relates to a comestible product, for example a food product comprising a composition of the invention, for example a dairy or non-dairy product, a solid food or a beverage, a food additive or supplement. The dairy product may be a milk, a cheese, or yoghurt. In one embodiment, the food product is a snack bar. The food product may comprise any amount of the composition of the invention, for example from 0.1% to 30% (w/w).

[0030] The food product may be a Food for Specific Medicinal Purposes (FSMP) which is defined as foods that are specifically formulated, processed and intended for the dietary management of diseases, disorders or medical conditions of individuals who are being treated under medical supervision. These foods are intended for the exclusive or partial feeding of people whose nutritional requirements cannot be met by normal foods.

[0031] In this specification, the term "Metabolic disorder" should be understood to include pre-diabetes, diabetes; Type-1 diabetes; Type-2 diabetes; metabolic syndrome; obesity; diabetic dyslipidemia; hyperlipidemia; hypertension; hypertriglyceridemia; hyperfattyacidemia; hypercholerterolemia; hyperinsulinemia, MODY, and HNF1A-MODY.

[0032] The invention also provides a pharmaceutical composition comprising a peptide or composition of the invention in combination with a suitable pharmaceutical carrier. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the peptide(s) or composition is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like.

[0033] The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

Brief Description of the Figures

[0034]

Figure **1.***Mean change in glucose (delta change, mmol/L) over 120 minutes post treatment with sodium caseinate or LFC 24.* Data presented based on n=62 healthy adults where error bars represent standard error of the mean (SEM). Treatment effect significant: p = 0.034.

Figure **2.***Mean change in insulin (delta change, mU/L) over 120 minutes post treatment with sodium caseinate or LFC 24.* Data presented based on n=62 healthy adults where error bars represent standard error of the mean (SEM). Treatment effect significant: p = 0.042.

Figure 3.*Mean change in GIP (delta change, pg/ml) over 120 minutes post treatment with sodium caseinate or hydrolysed casein (LFC 24).* Treatment effect significant: p = 0.031 Data presented based on n=62 healthy adults where error bars represent standard error of the mean. There was a significant increase in GIP (glucagon-like peptide) whereby treatment with LFC24 caused concentrations to rise 17.1% above the control levels at the 15 minute time point for example, which was an increase of 25.95pg/ml.

Figure 4.*Mean change in Non-Esterified Fatty Acids (delta change, mMol/L) over 120 minutes post treatment with sodium caseinate or LFC 24.* Data presented based on n=62 healthy adults where error bars represent standard error of the mean. Treatment effect significant: p< 0.001.Non-esterified fatty acids (NEFAs) were significantly reduced in response to consumption of LFC 24 compared to the intact sodium caseinate.

Figure 5.*Insulin secretion from BRIN BD11 cells following exposure to LFC24 (n=3/4).* Values are means ± SD. ** p<0.01. Cells are exposed to (1) 16.7 mM glucose (2) 16.7 mM glucose and 10 mM ala and (3) 16.7 mM glucose and LFC24 (1mg/ml).

Figure 6.*Insulin secretion from pancreatic beta cells* (*BRIN-BD11*). Insulin secretion from BRIN-BD11 cells following 2 hour incubation with 3T3-L1 adipocyte conditioned media and 3T3-L1 media with LFC24 (n=4). Exposure to LFC 24 prevented pancreatic beta cell dysfunction. ** p<0.01, *** p< 0.001

Figure *7A.Insulin secretion from pancreatic beta cells (BRIN BD11) (n=3/4) following exposure to 16.7 mmol/l glucose and the synthetic peptide No. 10.* Positive controls are 16.7 mM glucose and 16.7 mM glucose plus10 mM alanine. **** p<0.0001

Figure 7B.*Insulin secretion from pancreatic beta cells (BRIN BD11) (n=3/4) induced following exposure to 16.7 mmol/l glucose and the synthetic peptides.* Positive controls are 16.7 mM glucose and 16.7 mM glucose plus 10 mM alanine.

Figure 7C.*Insulin secretion from pancreatic beta cells (BRIN BD11) (n=3) following exposure to 16.7 mmol/l glucose and combination of the synthetic peptides.* Positive controls are 16.7 mM glucose and 16.7 mM glucose and 10 mM alanine. A pooled sample of peptides 1,3,4,5,9,10 and 11 was more potent than the combination of synthetic peptides 2, 6, 7 and 8.

Figure 8.*Acute treatment with LFC 24 had a glucose lowering effect during a glucose tolerance test in ob/ob mice 15 minutes (*p<0.05) and 120 minutes (*p<0.05) compared to the control treatment group.* Results are expressed as ±S.D. (Control n=5, treated n=6). Dashed line represents control group and solid line represent treatment with LFC24.

Figure 9.*Long term treatment with LFC 24 induced a significant reduction in the overall hepatic fat percentage in ob/ob mice following 12 weeks treatment. Control represents control treated mice and treated represent treatment with LFC24.* Treatment with a casein-derived hydrolysate caused a 43 % reduction in liver fat compared to the livers of control mice. Results are expressed as ±S.D of 6 separate 40 x images of each liver. (Control n=6, LFC24 treated n=5), (p<0.05). Long term treatment with LFC 24 caused a significant reduction in the average hepatic fat globule size in ob/ob mice following 12 weeks treatment. Average fat globule size was reduced from an average of 39 microns in control livers to 28 microns in treated livers. Results are expressed as means ±S.D of 6 separate 40x images of each liver. (Control n=6, LFC24 treated n=5), (*p<0.05).

Figure 10.*long term treatment with a* casein-derived *hydrolysate caused a significant reduction in the hepatic fat globule size and overall hepatic fat content in ob/ob mice following 12 weeks treatment.* A & B represent H&E stained liver sections from control ob/ob mice which received water for 12 weeks. C & D represent H&E stained liver sections from ob/ob mice treated with a LFC24. Scale = 300μm

Figure 11.*Glucose levels during a GTT.* Values are means ± SEM. LFC24 protects against high fat diet (HFD) induced glucose intolerance. Supplementation with LFC24 protected mice from HFD-induced glucose intolerance. The glycaemic response to the GTT was significantly lower in HFD+LFC24 fed mice, compared to HFD fed mice with significantly lower plasma glucose levels post glucose challenge (p<0.001, p<0.05). Also the GTT area under the curve (AUC) was significantly lower in HFD+LFC24 compared to HFD fed mice. This improvement of glycaemic control was independent of body weight as both HFDs caused equivalent weight gain.

Figure 12.*LFC24 protects against high fat diet (HFD)induced reduction of pAKT levels in adipose tissue.* Mice were injected with 1.5 U/kg insulin 15 mins prior to sacrificing. Adipose tissue was immediately snap-frozen. Levels of (A) phosphorylated AKT were determined by western blot analysis. (B) Densitometry analysis of pAKT levels were normalized to GAPDH and expressed as arbitrary units (AU). (**p<0.01 w.r.t Chow+Insulin; ++p<0.01 w.r.t HFD+Insulin) (n=10mouse/group)

Detailed Description of the Invention

Preparation of bovine casein-derived enzymatic hydrolysate (LFC24)

[0035] The casein fraction of bovine milk was subjected to proteolytic digestion with a gastro-intestinal protease enzyme preparation (trypsin, chymotrypsin and elastase) between the temperature range of 30°C-60°C. Upon completion of the reaction, the solution was heated to inactivate the enzyme, and the digest was evaporated and spray dried. The resulting powdered preparation was assayed for biological activity as described below and separated by reverse-phase solid-phase extraction (SPE) for peptide analysis.

Human Study

Study population

[0036] Healthy subjects were recruited in the Dublin region by poster and radio advertising. The study was approved by the University College Dublin Human Ethics Research Committee. Interested candidates were screened and, once accepted, provided written, informed consent for their participation. Inclusion criteria stated that subject were aged 40-65 years, with BMI> 25kg/m^2 and be free from disease, prescribed medication, pregnancy or lactation.

Table 1. Baseline characteristics of the study population (data shown represents mean and standard deviation (SD) of 62 subjects (32 f, 30 m)

	Mean	SD
Age (years)	53.6	6.5
BMI (kg/m^2)	31.3	4.6
Body Fat %	37.0	8.2
Waist Circumference(cm)	93.7	10.6
Systolic Blood Pressure (mmHg)	128.9	16.9
Diastolic Blood Pressure (mmHg)	82.3	8.3

Study design and intervention

[0037] The study was a randomised crossover design with ingestion of an intact sodium caseinate and a casein-derived hydrolysate comprising all of the peptides of SEQUENCE ID NO'S: 1 to 11 (hereafter "LFC24"). In-tact sodium caseinate was obtained from Kerry Ingredients plc.. Each protein beverage was prepared by reconstituting 12g dried LFC24 or intact sodium caseinate in 120ml mineral water (Ballygowan), giving a 10% w/v solution.

[0038] On arrival fasting subjects were instructed to consume the meal containing 75 g carbohydrate and protein beverage within ten minutes.. Blood samples were taken at time points t=0, 15, 30, 60, 90 and 120 minutes.

Statistics

[0039] Data were expressed as mean values ± SEM. A linear mixed model analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA) comparing response of the control and treatment groups for all analytes. A p-value of 0.05 or less was considered significant.

Results

[0040] A total of 30 males and 32 females successfully completed the study (Table 1). The glucose and insulin response were significantly different between the LFC24 and sodium caseinate groups (Figure 1 and Figure 2).

In vitro study

[0041] LFC24 can promote insulin secretion from pancreatic beta cells (Figure 5). Experiments were initially performed in a cell line and confirmed using primary islets (data not shown). The ability of LFC24 to rescue pancreatic beta cell function was examined using an adipocyte condition media-beta cell function model. Exposure to LFC24 rescued the beta cell insulin secretion ability (Figure 6).

[0042] A series of peptides were identified to be present in LFC24. Following identification of the peptides a series of synthetic peptides were synthesised. The ability to promote insulin secretion was tested and the results are shown in Figure 7.

Animal model study

[0043] Three animal studies were performed. Study 1 and 2 used the ob/ob mouse model and study 3 used a diet induced obesity mouse model.

Study 1 and 2

Acute effect of a casein-derived *hydrolysate (LFC24) on glucose and insulin levels during a glucose tolerance test*

[0044] To assess the acute effects of LFC 24 a GTT was performed in ob/ob and C57BL/6 wildtype mice. A glucose lowering effect was found in ob/ob mice, decreased levels of glucose was observed at 15 minutes (*$p<0.05$) and 120 minutes (*$p<0.05$) compared to the control ob/ob group (Figure 8). Insulin was measured during the GTT for ob/ob mice; treatment with the casein-derived hydrolysate one hour prior to the GTT had no effect on insulin levels at 0, 15 and 60 minutes compared to control conditions.

Effect of supplementation with a casein-derived *hydrolysate (LFC24) on liver fat*

[0045] Hepatic histological studies demonstrated that treatment with a casein-derived hydrolysate (LFC24) caused a significant ($p<0.05$) reduction in overall fat content in the liver with a significant ($p<0.05$) reduction in fat droplet size (Figure 9). Treatment with a casein-derived hydrolysate caused a 43% reduction in overall liver fat and a 28% reduction in the average fat globule size (Figure 9) compared to the livers of control mice. Visual observation of H&E stained images of liver reveal vivid changes in liver fat following treatment (Figure 10).

Study 3

[0046] Using a diet induced obesity model animals were treated for 12 weeks with LFC 24. Treatment with LFC24 in the context of high fat feeding improved glucose clearance during a GTT compared to high fat feeding alone. LFC24 had no effect during chow feeding.

[0047] The invention is not limited to the embodiments hereinbefore described which may be varied in construction and detail without departing from the spirit of the invention.

SEQUENCE LISTING

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<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 4

Leu Gln Ser Trp Met His Gln Pro His Gln Pro Leu Pro Pro Thr Val
1 5 10 15

Met Phe Pro Pro Gln Ser Val Leu Ser Leu Ser Gln Ser Lys
20 25 30

<210> 5
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 5

Pro Pro Gln Ser Val Leu Ser Leu Ser Gln Ser Lys
1 5 10

<210> 6
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
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<400> 6

Met His Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro
1 5 10 15

Gln Ser Val Leu
20

<210> 7
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence

<400> 7

Met His Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro
1 5 10 15

Gln Ser Val Leu Ser Leu Ser Gln Ser Lys
20 25

<210> 8
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence

<400> 8

Ser Trp Met His Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe
1 5 10 15

Pro Pro Gln Ser Val Leu
20

<210> 9
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
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<400> 9

Ser Trp Met His Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe
1 5 10 15

Pro Pro Gln Ser Val Leu Ser Leu Ser Gln Ser Lys
20 25

<210> 10
<211> 50
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<213> Artificial Sequence

<220>
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<400> 10

Tyr Pro Val Glu Pro Phe Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp
1 5 10 15

Val Glu Asn Leu His Leu Pro Leu Pro Leu Leu Gln Ser Trp Met His
20 25 30

```
Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro Gln Ser
        35                  40                  45

Val Leu
    50

<210>  11
<211>  56
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic peptide

<400>  11

Tyr Pro Val Glu Pro Phe Thr Glu Ser Gln Ser Leu Thr Leu Thr Asp
1               5                   10                  15

Val Glu Asn Leu His Leu Pro Leu Pro Leu Leu Gln Ser Trp Met His
            20                  25                  30

Gln Pro His Gln Pro Leu Pro Pro Thr Val Met Phe Pro Pro Gln Ser
        35                  40                  45

Val Leu Ser Leu Ser Gln Ser Lys
    50                  55
```

Claims

1. A peptide having 12 to 60 amino acids and including (a) a sequence of SEQUENCE ID NO: 11, or (b) a bioactive fragment of SEQUENCE ID NO: 11, for use in improving glycemic management in a mammal.

2. A peptide of Claim 1 for use of Claim 1, in which the bioactive fragment of SEQUENCE ID NO: 11 comprises a sequence of SEQUENCE ID NO: 1 or 5.

3. A peptide of Claim 1, for use of Claim 1, in which the peptide includes a sequence selected from SEQUENCE ID NO:s1 to 11.

4. A peptide of Claim 1 or 2, for use of Claim 1, wherein the improvement in glycaemic management comprises lowering post-prandial blood glucose levels in a mammal, increasing post-prandial insulin secretion in a mammal, or both.

5. A composition comprising a plurality of different peptides, in which each of the plurality of peptides consists of 12 to 60 amino acids and includes (a) a sequence of SEQUENCE ID NO: 11, or (b) a bioactive fragment of SEQUENCE ID NO: 11.

6. A composition as claimed in Claim 5 in which the bioactive fragment of SEQUENCE ID NO: 11 includes the sequence of SEQUENCE ID NO: 1 or 5.

7. A composition according to Claim 5 and comprising substantially all of peptides of SEQUENCE ID NO:s: 1 to 11.

8. A composition according to any of Claims 5 to 7 and comprising a bovine casein product derived hydrolysate.

9. A food product comprising a composition according to any of Claims 5 to 8.

10. A composition according to any of Claims 5 to 8, or a food product of Claim 9, for use in improving glycemic management in a mammal, or for use in lowering post-prandial blood glucose levels in a mammal.

11. A composition according to any of Claims 5 to 8, or a food product of Claim 9, for use in increasing post-prandial insulin secretion in a mammal.

12. A composition of according to any of Claims 5 to 8, or a food product of Claim 9, for use in preventing or treating inflammation or an inflammatory disease, a metabolic disorder, or obesity.

13. A peptide having 12-60 amino acids and including a sequence of SEQUENCE ID NO: 11 or a fragment thereof that includes the sequence of SEQUENCE ID NO: 1 or 5, for use in preventing or treating a condition selected from a metabolic disorder or obesity.

14. A pharmaceutical composition comprising the composition of any of Claims 5 to 8 in combination with a suitable pharmaceutical carrier.

15. A bovine casein product derived hydrolysate comprising substantially all of peptides of SEQUENCE ID NO:s: 1 to 11.

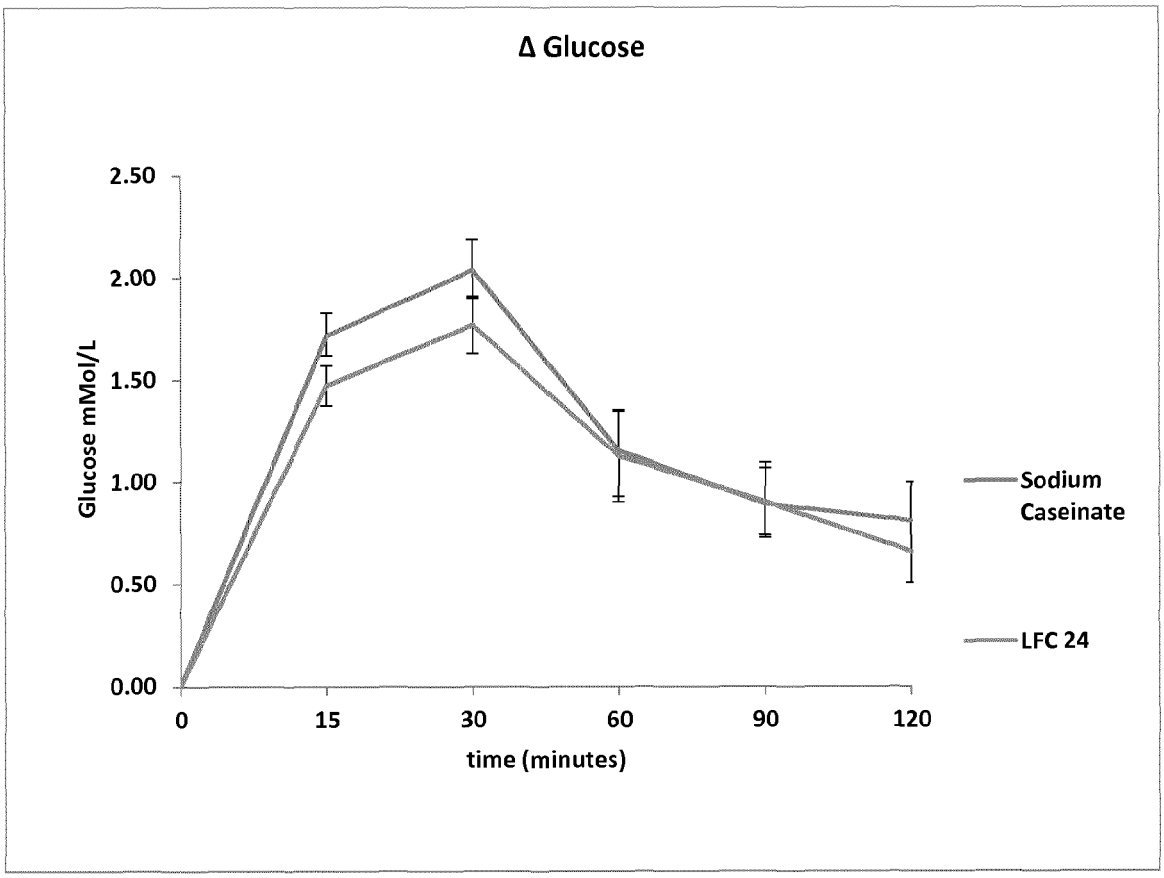
Δ Glucose
2.50
2.00
1.50
1.00
0.50
0.00
Glucose mMol/L
0
15
30
60
90
120
time (minutes)
Sodium Caseinate
LFC 24

Figure 1

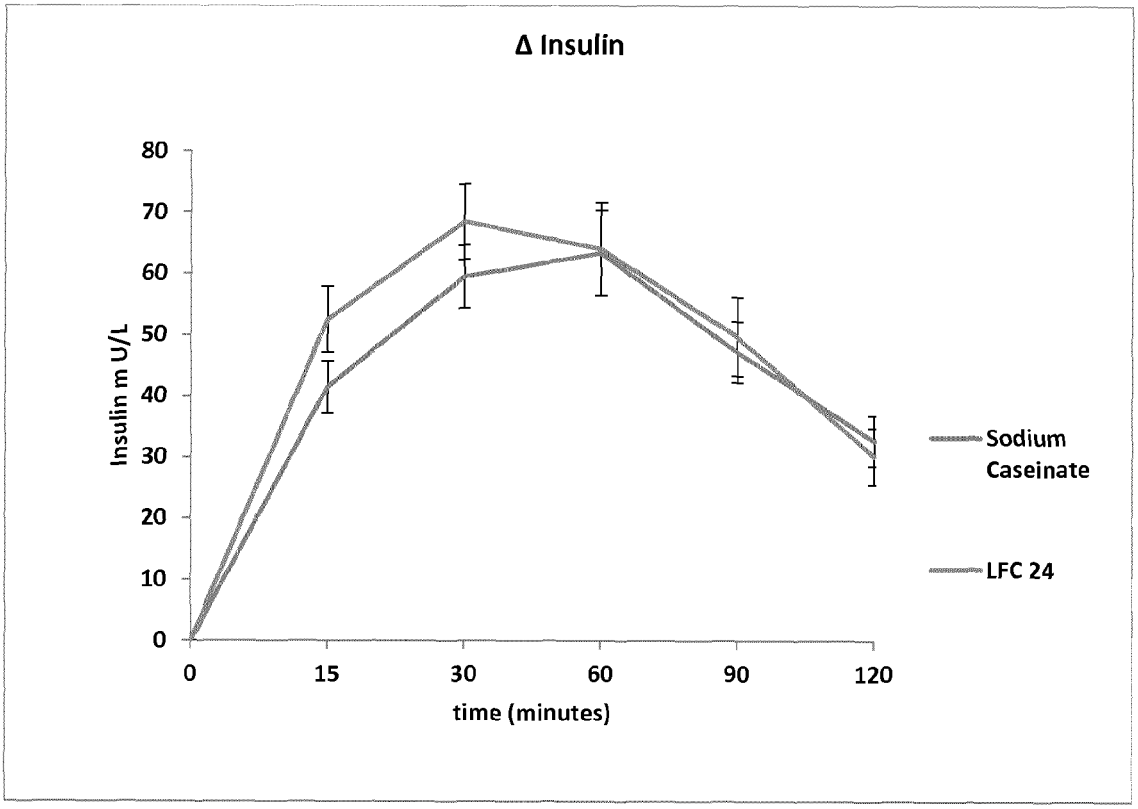
Δ Insulin
Insulin m U/L
80
70
60
50
40
30
20
10
0
0
15
30
60
90
120
time (minutes)
Sodium Caseinate
LFC 24

Figure 2

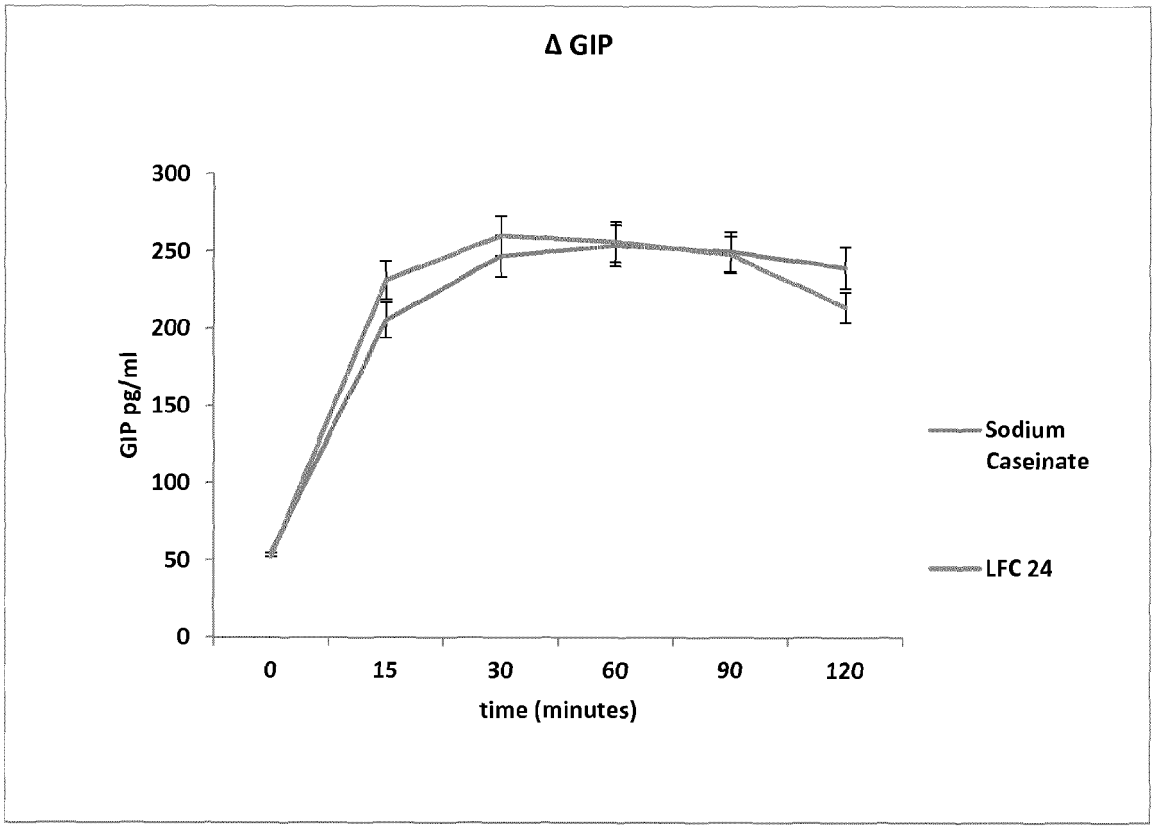
Δ GIP
300
250
200
150
100
50
0
GIP pg/ml
0
15
30
60
90
120
time (minutes)
Sodium Caseinate
LFC 24

Figure 3

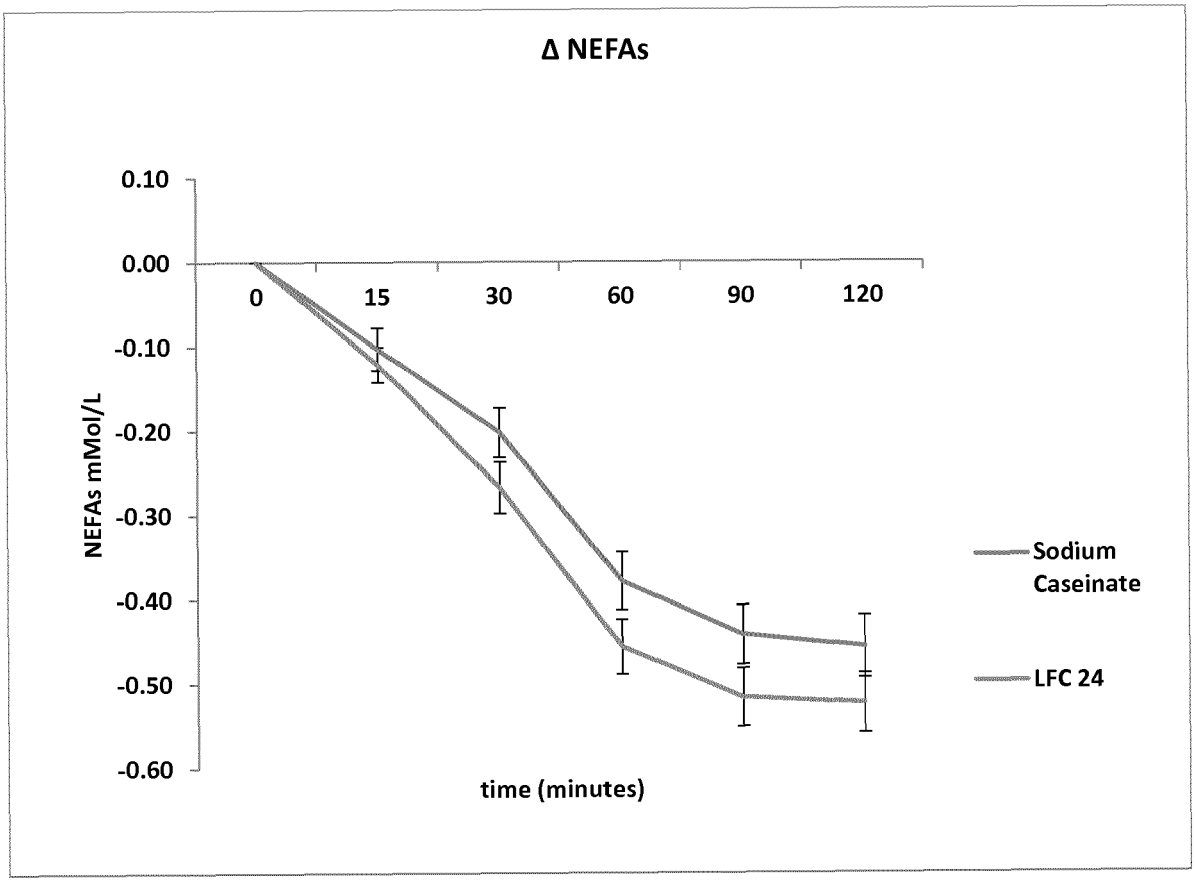
Δ NEFAs
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
-0.60
NEFAs mMol/L
0
15
30
60
90
120
time (minutes)
Sodium Caseinate
LFC 24

Figure 4

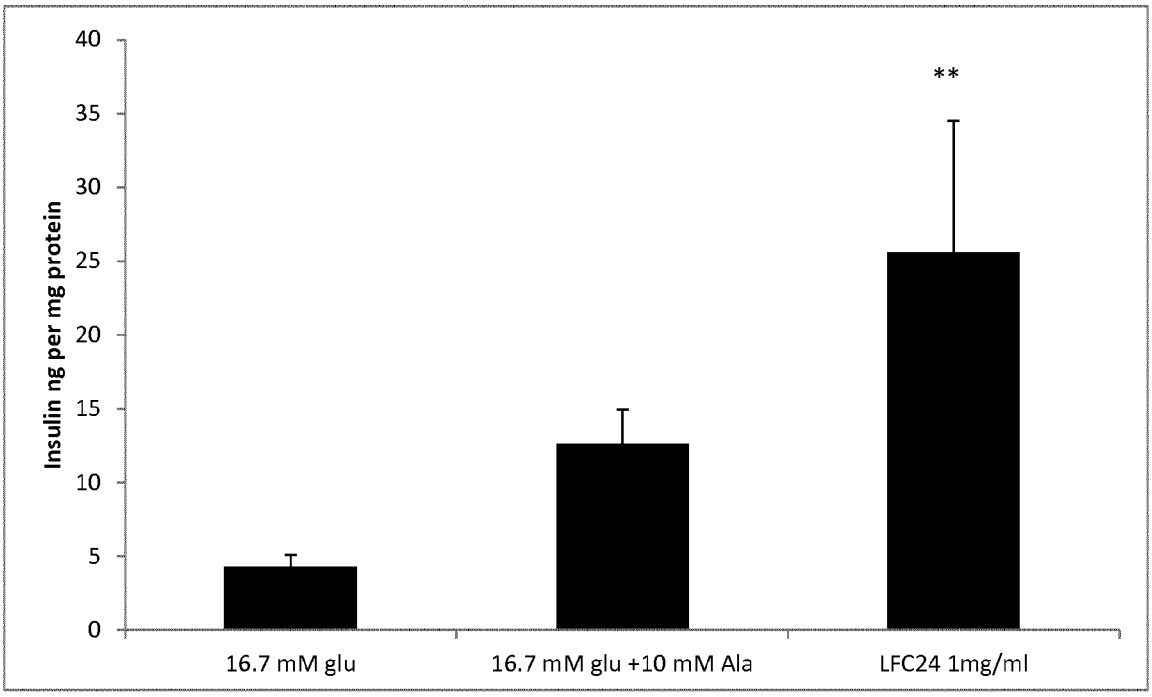
Insulin ng per mg protein
40
35
30
25
20
15
10
5
0
**
16.7 mM glu
16.7 mM glu +10 mM Ala
LFC24 1mg/ml

Figure 5

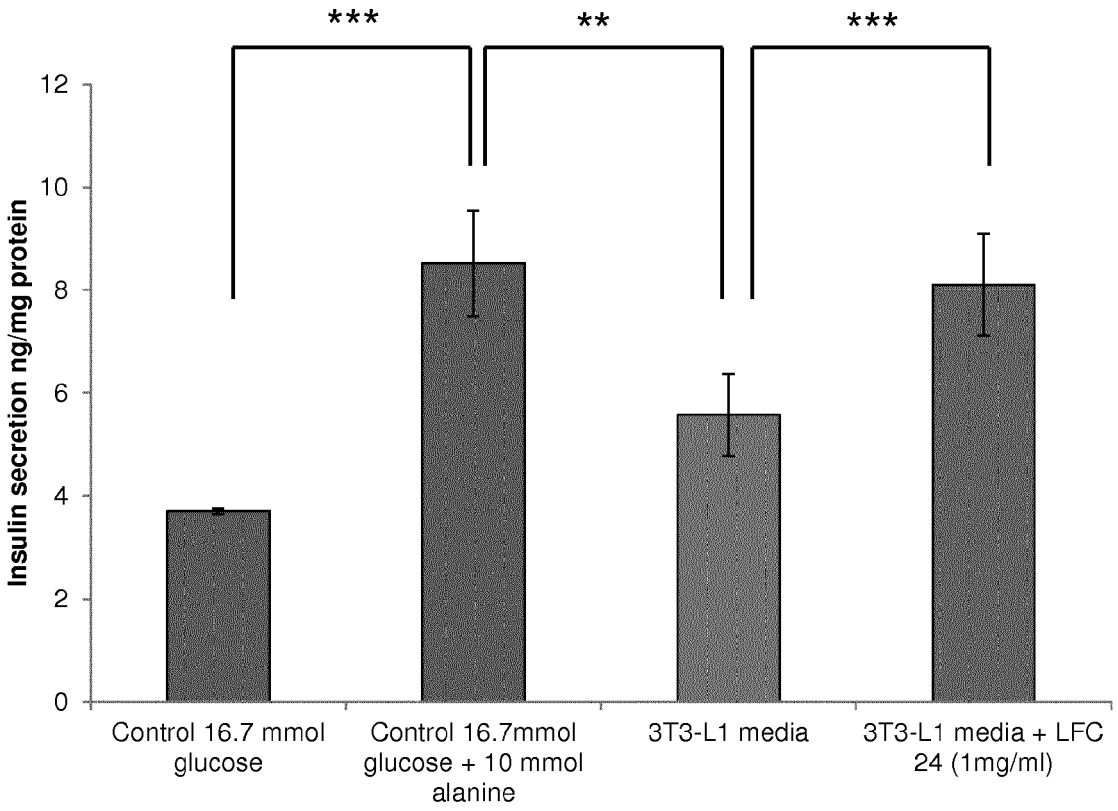
***
**
***
12
10
8
6
4
2
0
Insulin secretion ng/mg protein
Control 16.7 mmol glucose
Control 16.7mmol glucose + 10 mmol alanine
3T3-L1 media
3T3-L1 media + LFC 24 (1mg/ml)

Figure 6

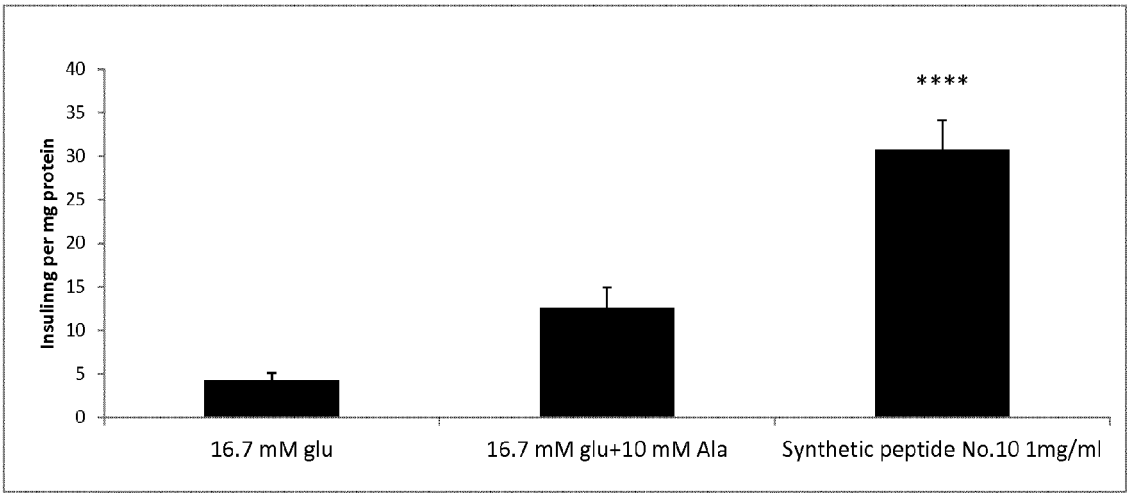

Figure 7A

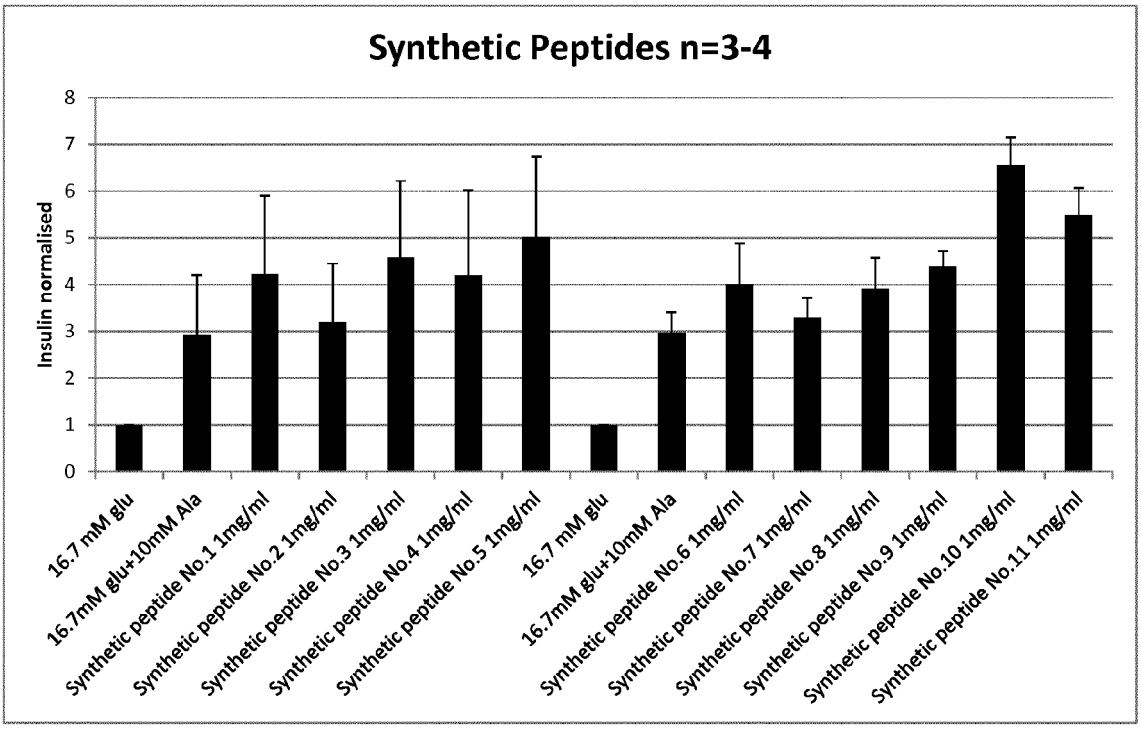

Figure 7B

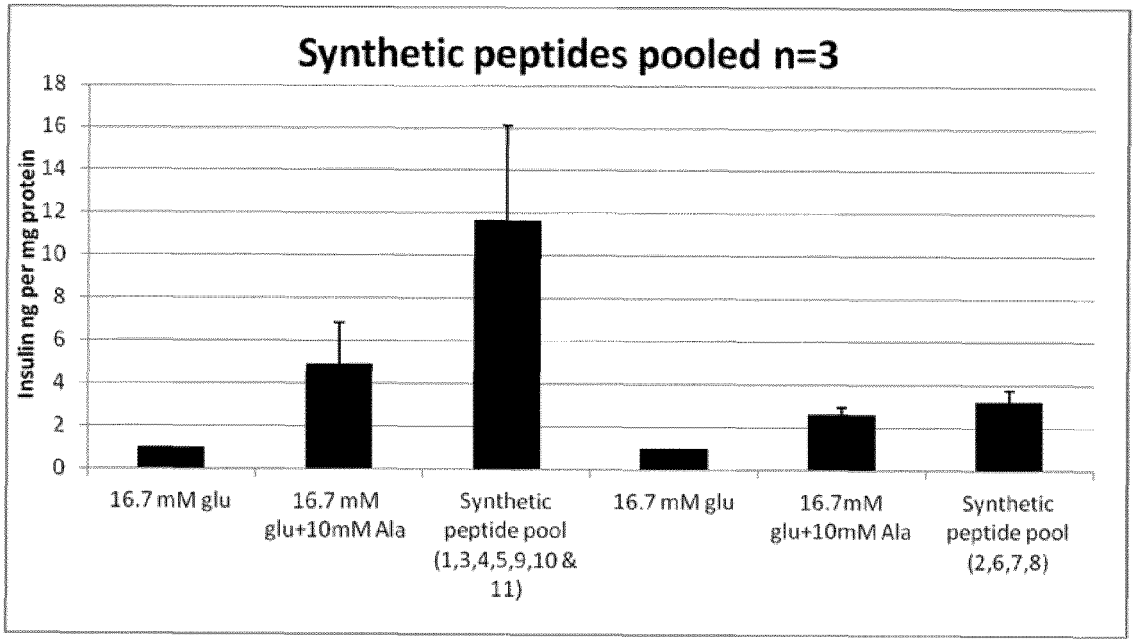
Synthetic peptides pooled n=3
Insulin ng per mg protein
18
16
14
12
10
8
6
4
2
0
16.7 mM glu
16.7 mM glu+10mM Ala
Synthetic peptide pool (1,3,4,5,9,10 & 11)
16.7 mM glu
16.7mM glu+10mM Ala
Synthetic peptide pool (2,6,7,8)

Figure 7C

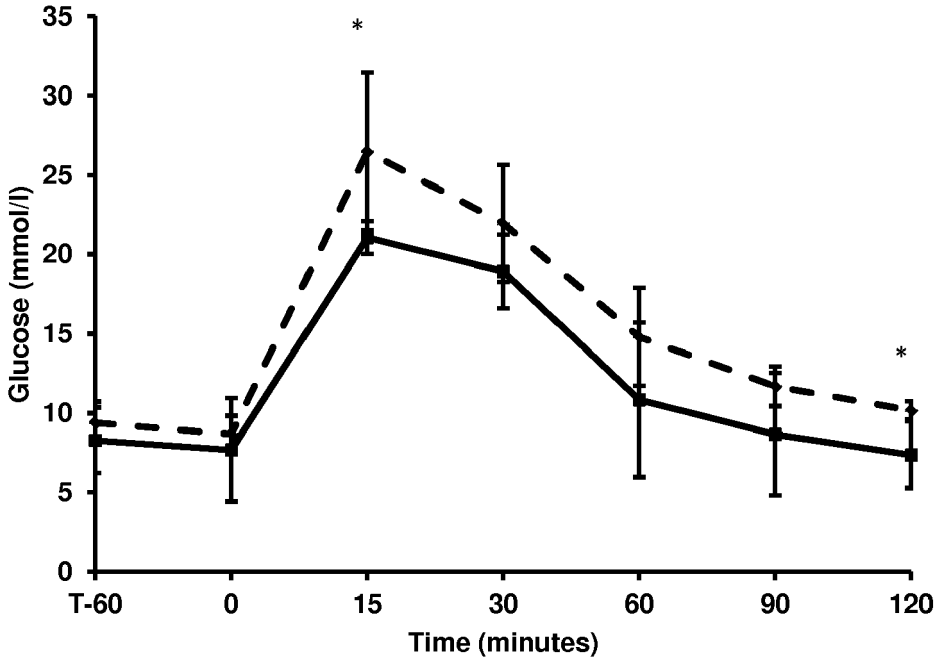
35
30
25
20
15
10
5
0
Glucose (mmol/l)
*
*
T-60
0
15
30
60
90
120
Time (minutes)

Figure 8

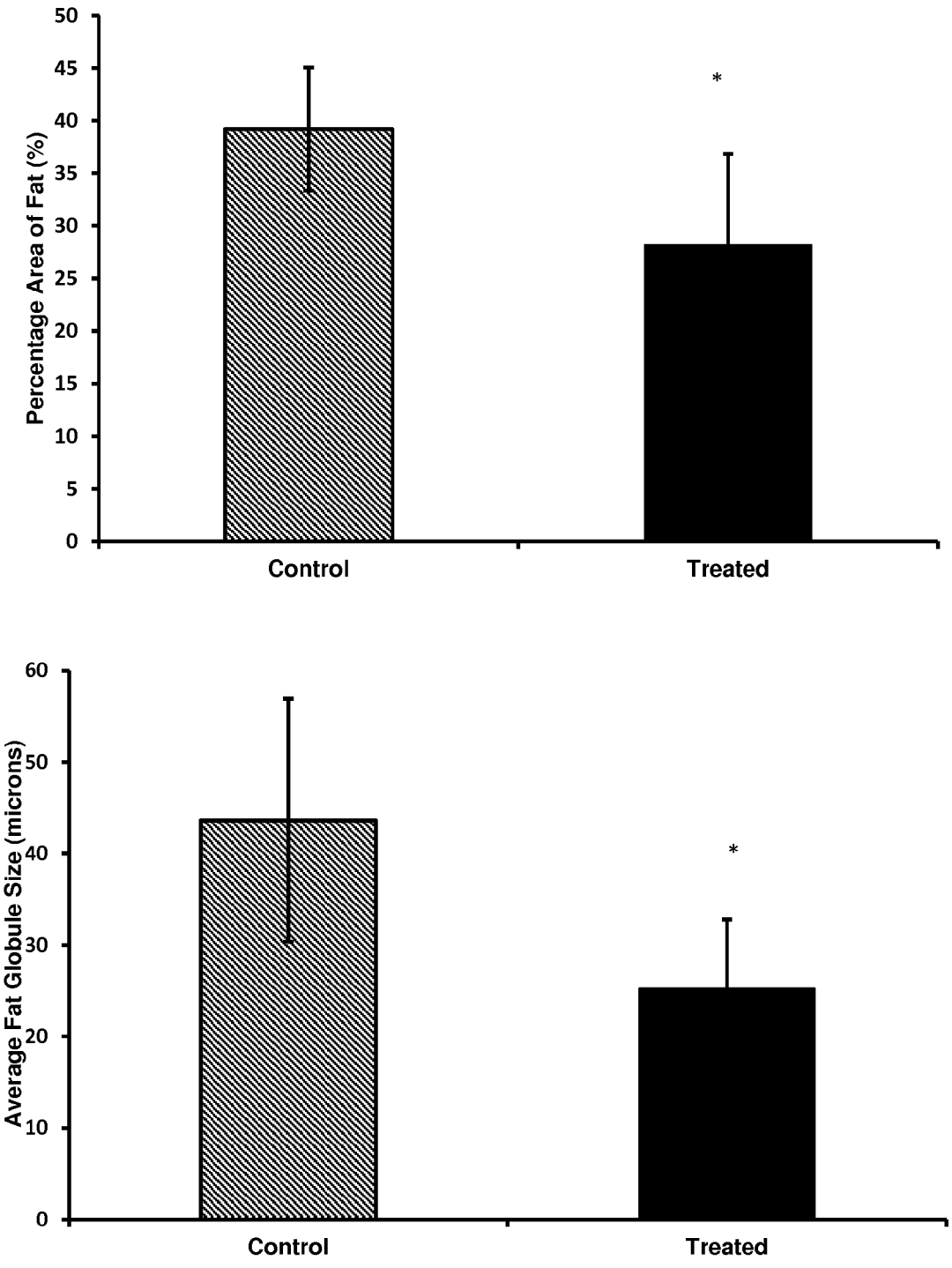
Percentage Area of Fat (%)
50
45
40
35
30
25
20
15
10
5
0
*
Control
Treated
Average Fat Globule Size (microns)
60
50
40
30
20
10
0
*
Control
Treated

Figure 9

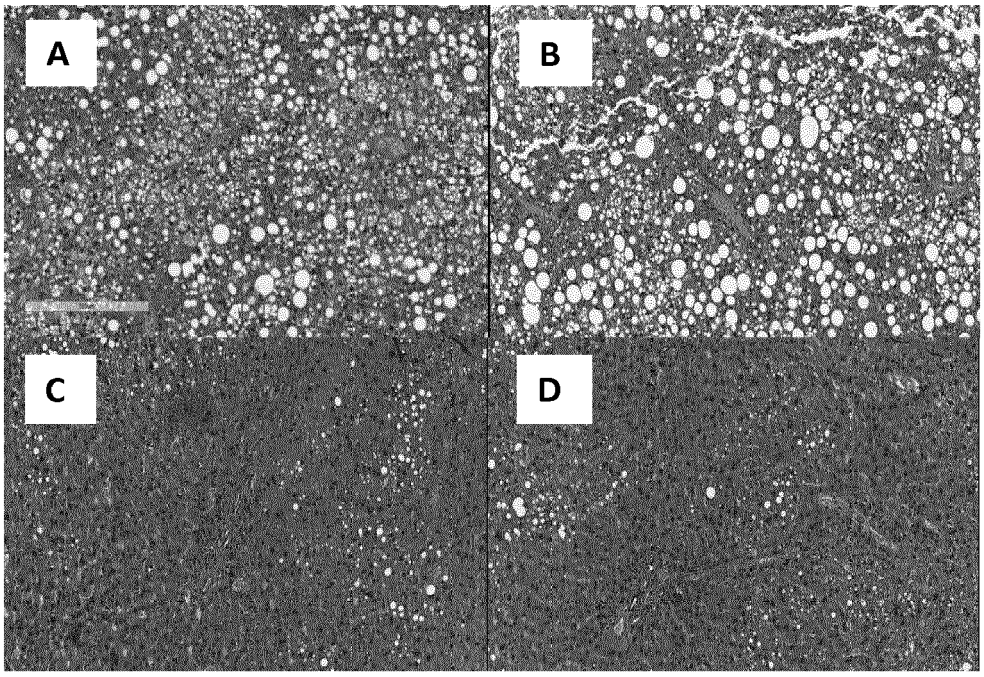

Figure 10

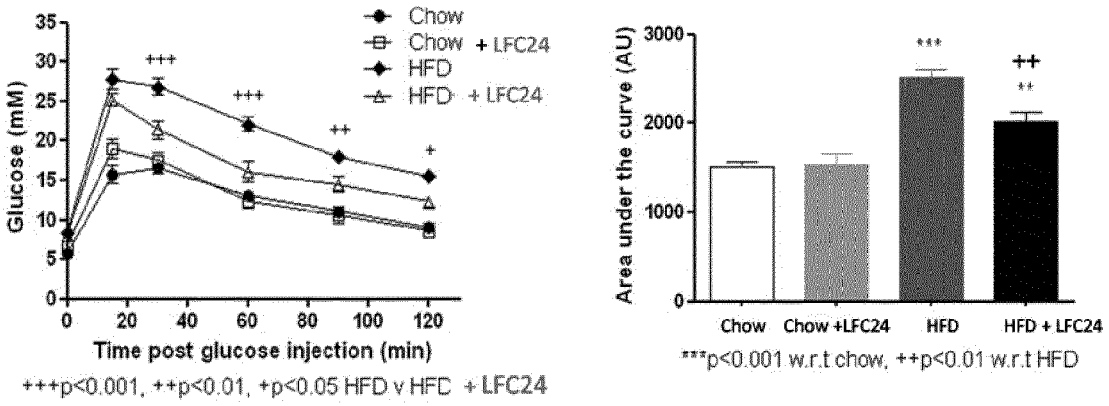
Chow
Chow + LFC24
HFD
HFD + LFC24
Glucose (mM)
Time post glucose injection (min)
+++p<0.001, ++p<0.01, +p<0.05 HFD v HFD + LFC24
Area under the curve (AU)
Chow
Chow +LFC24
HFD
HFD + LFC24
***p<0.001 w.r.t chow, ++p<0.01 w.r.t HFD

Figure 11

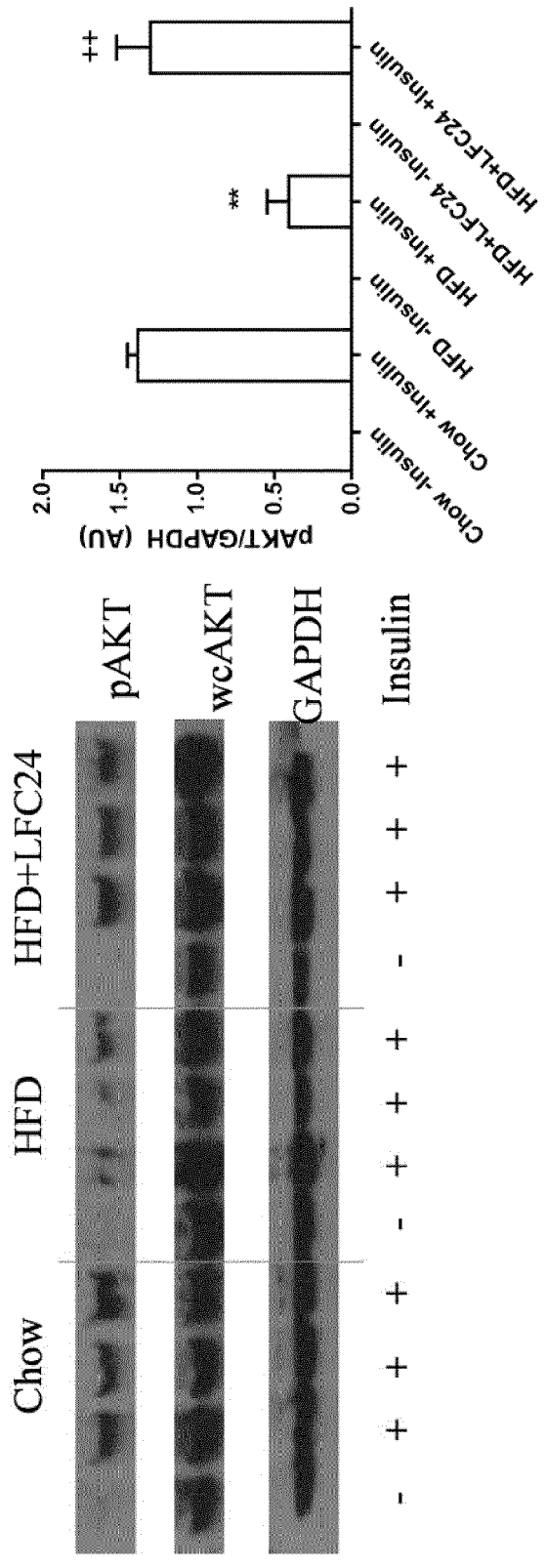
pAKT/GAPDH (AU)
2.0
1.5
1.0
0.5
0.0
Chow -insulin
Chow +insulin
HFD -insulin
HFD +insulin
HFD+LFC24 -insulin
HFD+LFC24 +insulin
++
**
pAKT
wcAKT
GAPDH
Insulin
HFD+LFC24
HFD
Chow
+ + + -
+ + + -
+ + + -

Figure 12

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Application Number
EP 14 16 7891

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 2005/089969 A1 (WISSLER JOSEF H [DE] ET AL) 28 April 2005 (2005-04-28) * examples 1-2; sequence 2 * -& DATABASE Geneseq [Online] 22 April 2004 (2004-04-22), "Insulin homologue exsulin peptide #2.", XP002730407, retrieved from EBI accession no. GSP:ADH92152 Database accession no. ADH92152 * the whole document * -& DE 101 49 668 A1 (NUMICO RES B V [NL]) 10 April 2003 (2003-04-10) * sequence 2 * -----	1-15	INV. A61K38/01 C07K7/08 C07K14/47
X	HERMITTE L ET AL: "Dietary protection against diabetes in NOD mice: Lack of a major change in the immune system", DIABETE & METABOLISME, MASSON, PARIS, FR, vol. 21, no. 4, 1 January 1995 (1995-01-01), pages 261-268, XP008118620, ISSN: 0338-1684 * page 262 * -----	1-15	TECHNICAL FIELDS SEARCHED (IPC) A61K C07K
X	WANG GEN-SHENG ET AL: "Hydrolysed casein diet protects BB rats from developing diabetes by promoting islet neogenesis", JOURNAL OF AUTOIMMUNITY, vol. 15, no. 4, December 2000 (2000-12), pages 407-416, XP002730174, ISSN: 0896-8411 * paragraph [introduction] * ----- -/--	1-15	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	30 September 2014	Mabit, Hélène

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D : document cited in the application
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	M.B. ESPECHE TURBAY ET AL: "[beta]-Casein hydrolysate generated by the cell envelope-associated proteinase of Lactobacillus delbrueckii ssp. lactis CRL 581 protects against trinitrobenzene sulfonic acid-induced colitis in mice", JOURNAL OF DAIRY SCIENCE, vol. 95, no. 3, 1 March 2012 (2012-03-01), pages 1108-1118, XP055119365, ISSN: 0022-0302, DOI: 10.3168/jds.2011-4735 * abstract *	5-9, 12-15	

X	WO 2012/176659 A1 (CALPIS CO LTD [JP]; UCHIDA NAOTO [JP]; GOTO HIROAKI [JP]; OHSAWA KAZUH) 27 December 2012 (2012-12-27) * the whole document * -& DATABASE Geneseq [Online] 20 June 2013 (2013-06-20), "Casein protein fragment, SEQ ID 16.", XP002730408, retrieved from EBI accession no. GSP:BAJ56696 Database accession no. BAJ56696 * the whole document * -& DATABASE Geneseq [Online] 20 June 2013 (2013-06-20), "Casein protein fragment, SEQ ID 4.", XP002730410, retrieved from EBI accession no. GSP:BAJ56684 Database accession no. BAJ56684 * the whole document * -/--	5-9,14, 15	TECHNICAL FIELDS SEARCHED (IPC)

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Place of search	Date of completion of the search	Examiner
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	-& DATABASE Geneseq [Online] 20 June 2013 (2013-06-20), "Casein protein fragment, SEQ ID 3.", XP002730434, retrieved from EBI accession no. GSP:BAJ56683 Database accession no. BAJ56683 * sequence * -& EP 2 735 616 A1 (CALPIS CO LTD [JP]) 28 May 2014 (2014-05-28) * paragraph [[0009]]; sequences 3, 4, 16 * -----		
X	JINSMAA Y ET AL: "Enzymatic release of neocasomorphin and beta-casomorphin from bovine beta-casein", PEPTIDES, ELSEVIER, AMSTERDAM, NL, vol. 20, no. 8, 1 January 1999 (1999-01-01), pages 957-962, XP002980615, ISSN: 0196-9781, DOI: 10.1016/S0196-9781(99)00088-1 * paragraphs [02.1] - [02.3] * -----	5-8,14, 15	TECHNICAL FIELDS SEARCHED (IPC)
X	WO 2006/026569 A2 (UNIV NORTHEASTERN [US]; WU SHIAW-LIN [US]; HANCOCK WILLIAM S [US]; KAR) 9 March 2006 (2006-03-09) * page 21 * -& DATABASE Geneseq [Online] 4 May 2006 (2006-05-04), "Bovine beta-casein peptide fragment (aa114-169).", XP002730411, retrieved from EBI accession no. GSP:AEG20987 Database accession no. AEG20987 * the whole document * ----- -/--	5-8,14, 15	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	30 September 2014	Mabit, Hélène

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Application Number
EP 14 16 7891

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	RONGXIN SU ET AL: "Pancreatic hydrolysis of bovine casein: Peptide release and time-dependent reaction behavior", FOOD CHEMISTRY, ELSEVIER LTD, NL, vol. 133, no. 3, 31 January 2012 (2012-01-31), pages 851-858, XP028468314, ISSN: 0308-8146, DOI: 10.1016/J.FOODCHEM.2012.01.103 [retrieved on 2012-02-08] * page 851, column 2, paragraph 2 * * paragraph [02.1] - paragraph [02.2]; table 3 *	5-8,14, 15	
A	SAAVEDRA L ET AL: "An overview of omic analytical methods applied in bioactive peptide", FOOD RESEARCH INTERNATIONAL, vol. 54, no. 1, 2013, pages 925-934, XP028750279, ISSN: 0963-9969, DOI: 10.1016/J.FOODRES.2013.02.034	1-15	TECHNICAL FIELDS SEARCHED (IPC)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP 2735616 A1	28-05-2014	EP 2735616 A1	28-05-2014
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		WO 2006026569 A2	09-03-2006

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Non-patent literature cited in the description

- **J. M. STEWART ; J. D. YOUNG.** Solid Phase Peptide Synthesis. Pierce Chemical Company, 1984 **[0024]**
- **M. BODANZSKY ; A. BODANZSKY.** The Practice of Peptide Synthesis. Springer Verlag, 1984 **[0024]**